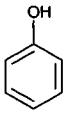
REFERENCE
Ferrara,S.D.; Tedeschi,L.; Frison,G.; Castagna,F. Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine, *J.Anal.Toxicol.*, **1992**, *16*, 217–222.

Phenol



Molecular formula: C_6H_6O
Molecular weight: 94.11
CAS Registry No.: 108-95-2
Merck Index: 7390

SAMPLE
Matrix: air
Sample preparation: Pull 5-150 L air through 10 mL 0.06% NaOH in water at 1-2 L/min. Add 1 mL buffer and 3 mL 0.1% 4-nitrobenzenediazonium tetrafluoroborate in water, make up to 20 mL with water, let stand for 15 min, inject a 2-40 μL aliquot. (Buffer was 0.51% sodium bicarbonate containing 0.21% sodium carbonate, pH 11.5.)

HPLC VARIABLES
Column: 200 × 4.6 Polygosil 60-5 C18 (Macherey-Nagel)
Mobile phase: MeOH:water 85:15
Flow rate: 1.1
Injection volume: 2-40
Detector: UV 365

CHROMATOGRAM
Retention time: 4
Limit of detection: 50 pg

OTHER SUBSTANCES
Also analyzed: o-cresol, m-cresol, p-cresol, 1-naphthol, 2-naphthol, 2,3-xylenol, 2,4-xylenol, 2,5-xylenol, 2,6-xylenol, 3,4-xylenol, 3,5-xylenol

KEY WORDS
derivatization

REFERENCE
Kuwata,K.; Uebori,M.; Yamazaki,Y. Determination of phenol in polluted air as *p*-nitrobenzeneazophenol derivative by reversed phase high performance liquid chromatography, *Anal.Chem.*, **1980**, *52*, 857–860.

SAMPLE
Matrix: air
Sample preparation: Pull 5-150 L air through 10 mL 0.12% NaOH in water at 1-2 L/min. Remove a 5 mL aliquot, add 1 mL buffer, add 3 mL 0.1% 4-nitrobenzenediazonium tetrafluoroborate in water, make up to 20 mL with water, let stand for 30 min, add 1 mL 1% NaOH, add 1 mL carbon tetrachloride, shake, centrifuge, inject a 2-40 μL aliquot of the aqueous (p-unsubstituted phenols) layer or a 2-10 μL aliquot of the organic (p-substituted phenols) layer. (Buffer was 0.51% sodium bicarbonate containing 0.21% sodium carbonate, pH 11.5.)

HPLC VARIABLES
Column: 200 × 4.6 5 μm LiChrosorb RP-18
Mobile phase: MeOH:water 85:15
Flow rate: 1.1
Injection volume: 2-40
Detector: UV 365

CHROMATOGRAM
Retention time: 3.89

Limit of detection: 0.05 ng

OTHER SUBSTANCES
Simultaneous: o-chlorophenol, m-chlorophenol, p-chlorophenol, o-cresol, m-cresol, p-cresol, o-ethylphenol, m-ethylphenol, p-ethylphenol, α-naphthol, β-naphthol, 2,3-xylenol, 2,4-xylenol, 2,5-xylenol, 2,6-xylenol, 3,4-xylenol, 3,5-xylenol

KEY WORDS
derivatization

REFERENCE
Kuwata,K.; Uebori,M.; Yamazaki,Y. Reversed-phase liquid chromatographic determination of phenols in auto exhaust and tobacco smoke as *p*-nitrobenzeneazophenol derivatives, *Anal.Chem.*, **1981**, *53*, 1531–1534.

SAMPLE
Matrix: air
Sample preparation: Condition a Sep Pak silica SPE cartridge with 10 mL dichloromethane and dry with helium at 5 L/min. Pull air through a 0.80 μm cellulose ester membrane filter and the SPE cartridge at 2 L/min for 1 h, desorb the filter with 5 mL 1% acetic acid with sonication for 10 min, elute the SPE cartridge with 5 mL 1% acetic acid, inject aliquots of the eluates.

HPLC VARIABLES
Guard column: 30 × 4.6 Spheri-5 RP-18
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was 1% acetic acid. B was MeCN:acetic acid 99:1. A:B from 0:100 to 90:10 over 10.5 min, to 78:22 to 24.5 min, to 0:100 (step gradient), maintain at 0:100 for 5 min, re-equilibrate for 12 min.
Flow rate: 2
Injection volume: 200
Detector: F ex 304 em 338 for 6.3 min, ex 280 em 325 for 7.7 min, ex 257 em 330 for 5.3 min, ex 342 em 464 for 4.7 min, ex 285 em 310 for 11 min

CHROMATOGRAM
Retention time: 15.5
Limit of detection: 0.29 μg/cu.m.

OTHER SUBSTANCES
Simultaneous: catechol, cresol, hydroquinone, 3-methylcatechol, scopoletin

KEY WORDS
SPE

REFERENCE
Risner,C.H. The quantification of hydroquinone, catechol, phenol, 3-methylcatechol, scopoletin, m+p-cresol and o-cresol in indoor air samples by high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4117–4140.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 10 μL 100 μg/mL p-ethylphenol in water, acidify to pH 1.0 with 1 M HCl, saturate with 100 mg NaCl, add 300 μL ethyl acetate, shake for 10 min, centrifuge at 1300 g for 5 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 Finepak Sil C18S (Jasco)
Mobile phase: MeCN:water 30:70
Flow rate: 1
Injection volume: 10
Detector: F ex 260 em 305 or MS, Hitachi M-1000S quadrupole, APCI, desolvation 399°, vaporization 300°, drift voltage 40 V, negative-ion mode, m/z = 93

CHROMATOGRAM
Retention time: 5.8
Internal standard: p-ethylphenol (m/z = 121) (16)
Limit of detection: <1 μM

OTHER SUBSTANCES
Extracted: p-cresol

KEY WORDS
serum

REFERENCE
Niwa,T. Phenol and p-cresol accumulated in uremic serum measured by HPLC with fluorescence detection, *Clin.Chem.*, **1993**, *39*, 108–111.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 272

CHROMATOGRAM
Retention time: 3.40
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclam-

ide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.422

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: bulk, formulations

Sample preparation: Bulk. Weigh out 100 mg morphine, dissolve in 25 mL MeOH:water:acetic acid 24:72:1, dilute with MeOH:water:acetic acid 24:72:1 to a final concentration of 240 μg/mL morphine, filter (0.45 μm), inject a 20 μL aliquot. Injections. Dilute with MeOH:water:acetic acid 24:72:1 to a final concentration of 240 μg/mL morphine, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:5 mM sodium 1-heptanesulfonate:acetic acid 24:72:1

Flow rate: 1.5
Injection volume: 20
Detector: UV 284

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: morphine, 2-mercaptobenzothiazole (UV 230), pseudomorphine (UV 230)

KEY WORDS
injections

REFERENCE
Bello,A.C.; Jhangiani,R.K. Liquid chromatographic determination of morphine sulfate and some contaminants in injections and bulk drug material: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1046–1048.

SAMPLE
Matrix: feces, urine
Sample preparation: Urine. 5 mL Urine + 2 mL concentrated HCl + 1 mL 250 μg/mL p-chlorophenol in water, boil for 1 h, cool, add 4 mL diethyl ether, extract by repeated inversion for 1 min, centrifuge at 500 g for 10 min. Remove the organic layer and add it to 3 mL 50 mM NaOH in MeOH, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 500 μL water, filter (0.45 μm), inject a 50 μL aliquot of the filtrate. Feces. 500 mg Feces + 5 mL 100 mM pH 5.5 phosphate buffer + 50 μL 250 μg/mL p-chlorophenol in water, vortex, centrifuge at 500 g for 10 min. Remove the top layer and add it to 2 mL concentrated HCl, boil for 1 h, cool, add 4 mL diethyl ether, extract by repeated inversion for 1 min, centrifuge at 500 g for 10 min. Remove the organic layer and add it to 3 mL 50 mM NaOH in MeOH, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 250 μL water, filter (0.45 μm), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Econosil RP-18
Mobile phase: MeOH:20 mM pH 4.0 phosphate buffer 48:52
Flow rate: 0.7
Injection volume: 50
Detector: UV 270

CHROMATOGRAM
Retention time: 14
Internal standard: p-chlorophenol (37)
Limit of detection: 200 ng

OTHER SUBSTANCES
Extracted: p-cresol

REFERENCE
Birkett,A.M.; Jones,G.P.; Muir,J.G. Simple high-performance liquid chromatographic analysis of phenol and p-cresol in urine and feces, *J.Chromatogr.B*, **1995**, *674*, 187–191.

SAMPLE
Matrix: formulations
Sample preparation: Dilute a 1 mL aliquot to a theoretical concentration of 30 μg/mL with MeCN:water 2:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 mm long 5 μm Waters Resolve C18
Mobile phase: MeOH:100 mM ammonium acetate:diethylamine 1280:2720:4, adjusted to a pH of 7.5
Column temperature: 30
Flow rate: 1

Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: nizatidine sulfoxide, nizatidine amide, nizatidine

KEY WORDS
injections

REFERENCE
Raineri,D.L.; Cwik,M.J.; Rodvold,K.A.; Deyo,K.L.; Scaros,L.P.; Fischer,J.H. Stability of nizatidine in commonly used intravenous fluids and containers, *Am.J.Hosp.Pharm.*, **1988**, *45*, 1523–1529.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 25 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb C18
Mobile phase: MeOH:THF:isopropanol:water 30:3.5:1.5:65
Column temperature: 40
Flow rate: 1
Injection volume: 25
Detector: UV 245

CHROMATOGRAM
Retention time: 17

OTHER SUBSTANCES
Simultaneous: methylparaben, propylparaben, flumazenil
Noninterfering: aminophylline, cimetidine, dobutamine, dopamine, famotidine, lidocaine, procainamide, ranitidine

KEY WORDS
injections; 5% dextrose

REFERENCE
Olsen,K.M.; Gurley,B.J.; Davis,G.A.; Christensen,R.; Monaghan,M.S. Stability of flumazenil with selected drugs in 5% dextrose injection, *Am.J.Hosp.Pharm.*, **1993**, *50*, 1907–1912.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 12.5 × 4 5 μm Zorbax RX-C18
Column: 250 × 4.6 5 μm Zorbax RX-C18
Mobile phase: MeCN:buffer 20:80 (Buffer was 50 mM NaH_2PO_4 + 1 mM tetramethylammonium chloride + 0.5 mM 1-octanesulfonic acid adjusted to pH 3.5 with concentrated orthophosphoric acid.)
Column temperature: 25
Flow rate: 1
Injection volume: 20
Detector: UV 203

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: atropine, tropic acid, obidoxime, HI-6
Also analyzed: pralidoxime chloride

KEY WORDS
nerve agent antidote mixtures

REFERENCE
Paddle,B.M.; Dowling,M.H. Simple high-performance liquid chromatographic method for assessing the deterioration of atropine-oxime mixtures employed as antidotes in the treatment of nerve agent poisoning, *J.Chromatogr.*, **1993**, *648*, 373–380.

SAMPLE
Matrix: formulations
Sample preparation: Injections and ophthalmic solutions. Dilute with water to an atropine concentration of 80 μg/mL, inject a 20 μL aliquot. Ointment. Weigh out ointment equivalent to about 4 mg atropine sulfate, add 10 mL THF:water 80:20, sonicate and swirl until the ointment is completely dispersed, make up to 50 mL with water, filter (0.45 μm), inject a 20 μL aliquot

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: MeCN:50 mM NaH_2PO_4 10:90, pH adjusted to 4.0 with 10% phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: atropine, tropic acid
Noninterfering: benzyl alcohol, methylparaben, benzalkonium chloride, chlorobutanol

KEY WORDS
injections; ophthalmic solutions; ointments

REFERENCE
Lehr,G.J.; Yuen,S.M.; Lawrence,G.D. Liquid chromatographic determination of atropine in nerve gas antidotes and other dosage forms, *J.AOAC Int.*, **1995**, *78*, 339–343.

SAMPLE
Matrix: honey
Sample preparation: Condition a 3 mL Baker-10 C18 SPE cartridge with 3 mL MeOH and 3 mL water. Dissolve 10 g honey in 90 mL water, add 30 g NaCl, add 2 mL 10% phosphoric acid, distil about 30 mL at about 5 mL/min. Add a 15 mL aliquot of the distillate to 6 g NaCl and 5 mL 5% sodium bicarbonate, extract with 5 mL benzene (Caution! Benzene is a carcinogen!). Wash the organic layer with 3 mL 1% sodium bicarbonate, extract with 3 mL 100 mM NaOH, extract with 2 mL 100 mM NaOH. Combine the aqueous extracts and adjust the pH to 3.0 with 0.33 mM phosphoric acid, add 4.5 g NaCl, add to the SPE cartridge, let cartridge dry under vacuum for 3 min, elute with 1 mL MeOH, inject a 10 μL aliquot of the eluate.

HPLC VARIABLES
Guard column: 70 × 2 30 μm Co-Pell ODS
Column: 150 × 4.6 5 μm Inertsil ODS
Mobile phase: MeCN:buffer 20:80 (Buffer was 10 mM NaH_2PO_4 containing 2 mM EDTA adjusted to pH 5.0.)
Flow rate: 1
Injection volume: 10
Detector: E, Irica Model E-520, glassy carbon electrode 0.7 V, Ag/AgCl reference electrode or UV 270

CHROMATOGRAM
Retention time: 11
Limit of detection: 2 ppb

KEY WORDS
SPE

REFERENCE
Takeba,K.; Matsumoto,M.; Shida,Y.; Nakazawa,H. Determination of phenol in honey by liquid chromatography with amperometric detection, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 602–604.

SAMPLE
Matrix: perfusate
Sample preparation: 100 μL Perfusate (Kreb's Henselit buffer containing 1% bovine serum albumin) + 200 μL 5 μg/mL p-cresol in MeOH, vortex for 20 s, centrifuge at 10000 g for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 4.6 5 μm Spherisorb C18
Mobile phase: MeOH:water:orthophosphoric acid 40:60:0.1, pH 2.7 ± 0.02
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5
Internal standard: p-cresol (10.3)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: phenyl-β-D-glucuronide

REFERENCE
Thompson,M.J.; Ballinger,L.N.; Cross,S.E.; Roberts,M.S. High-performance liquid chromatographic determination of phenol, 4-nitrophenol, β-naphthol and a number of their glucuronide and sulphate conjugates in organ perfusate, *J.Chromatogr.B*, **1996**, *677*, 117–122.

SAMPLE
Matrix: solutions
Sample preparation: Mix solution with 5 mL 200 g/L sodium acetate trihydrate and 650 μL reagent, let stand for 1 min, add 5 mL 160 g/L sodium carbonate monohydrate, add 150 mg tetrabutylammonium bromide, extract with 2 mL n-butanol, centrifuge, inject an aliquot of the organic layer. (Prepare reagent by mixing 2.566 g p-aminobenzonitrile and 108 mL concentrated HCl in 1 L water. Cool a 25 mL aliquot in an ice bath, slowly add with stirring 3 mL of a 25 g/L sodium nitrite solution. Use reagent within 1 h.)

HPLC VARIABLES
Column: 250 × 2.6 HC ODS/SIL-X C18 (Perkin-Elmer)
Mobile phase: MeOH:water 64:36
Flow rate: 1
Injection volume: 10
Detector: UV 370

CHROMATOGRAM
Retention time: 3.20
Limit of detection: 10 ppb

OTHER SUBSTANCES
Simultaneous: 2-sec-butylphenol, 2-tert-butylphenol, 3-tert-butylphenol, catechol, o-cresol, m-cresol, p-cresol, 2,3-dimethylphenol, 2-5-dimethylphenol, 2,6-dimethylphenol, 3,5-dimethylphenol, 3-hydroxybenzoic acid, 3-nitrophenol, salicylic acid, 2,3,5,6-tetramethylphenol, 3-trifluoromethylphenol

KEY WORDS
derivatization

REFERENCE
Baiocchi,C.; Campi,E.; Gennaro,M.; Mentasti,E.; Mirti,P. Reversed phase liquid chromatographic separation of phenolic compounds with a new derivatizing reaction, *Chromatographia*, **1982**, *15*, 660–664.

SAMPLE
Matrix: solutions
Sample preparation: Mix 10 mL of a 50 μg/mL solution with 2 mL 100 mg/mL NaOH and 5 mL reagent, mix, let stand for 15 min, add 3.4 mL 15 mg/mL tetrabutylammonium bromide in butanol (saturated with water), extract, inject a 10 μL aliquot of the organic layer. (Prepare reagent by mixing 5 volumes 7.6 mg/mL sulfanilic acid with 1 volume 470 mg/mL sulfuric acid, cool in an ice bath, slowly add 5 volumes 3.4 mg/mL sodium nitrite. Discard the reagent after 10 min.)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak RP phenyl
Mobile phase: MeOH:water 48:52 containing 3 mM tetrabutylammonium bromide
Flow rate: 2
Injection volume: 10
Detector: UV 370

CHROMATOGRAM
Retention time: 5.12
Limit of quantitation: 100 ppb

OTHER SUBSTANCES
Simultaneous: 2-sec-butylphenol, 3-t-butylphenol, 3-chlorophenol, 2-methylphenol, 3-methylphenol, 4-nitrophenol, 2,3-xylenol, 2,5-xylenol, 2,6-xylenol, 3,5-xylenol
Noninterfering: 2-t-butylphenol, 4-methylphenol

KEY WORDS
derivatization

REFERENCE
Baiocchi,C.; Gennaro,M.C.; Campi,E.; Mentasti,E.; Aruga,R. HPLC identification and separation of phenolic compounds derivatized with diazotized sulfanilic acid. Structural effects on retention times, *Anal.Lett.*, **1982**, *15*, 1539–1548.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb RP 18
Mobile phase: MeOH:10 mM pH 5.5 potassium phosphate buffer 3.5:96.5
Flow rate: 2-3
Detector: UV 254

CHROMATOGRAM
Retention time: 27

OTHER SUBSTANCES
Simultaneous: catechol, hydroquinone (quinol), phenyl glucuronide, phenyl glucoside, phenyl galactopyranoside, phenyl sulfate, resorcinol

REFERENCE
Beyer,J.; Frank,G. Hydroxylation and conjugation of phenol by the frog *Rana temporaria*, *Xenobiotica*, **1985**, *15*, 277–280.

SAMPLE

Matrix: solutions

Sample preparation: Mix 3.5 mL of a 40 nM-50 μM solution in benzene with 500 μL 3 mM reagent in benzene and 100 μL 3.7 mM pyridine in benzene (Caution! Benzene is a carcinogen!), heat in the dark at 100° for 40 min (primary and secondary alcohols) or at 140° for 50 min (tertiary alcohols), cool, dilute 100-fold with mobile phase, inject a 20 μL aliquot. (The reagent is 2-(5-chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran (Dojindo Laboratories, Kumamoto, Japan). Synthesis is as follows. Add ethyl oxalyl chloride in ether to a solution of diazomethane in ether at 0° to give ethyl diazopyruvate (Caution! Diazo compounds are explosive and toxic!) (cf. Buehler,C.A.; Pearson,D.E. Survey of Organic Syntheses, Wiley, New York, 1970, p. 179). Heat 100 mg ethyl diazopyruvate, a few mg copper(II) acetylacetonate, and 400 μL chloroacetonitrile in benzene at 60° overnight (Caution! Benzene is a carcinogen!), cool, add to sodium bicarbonate solution, extract with ether, dry the organic layer, evaporate, chromatograph on silica with petroleum ether:ethyl acetate 90:10, distil the product at 90°/12 mm Hg to give ethyl 2-chloromethyl-5-oxazolecarboxylate as an oil in 18% yield (US Patent 4 603 209 (July 29, 1986)). Add 2 mL phosphorus oxychloride dropwise to a solution of 2 g sesamol in 3 mL DMF at 0°, heat on a steam bath with frequent shaking for 1 h, cool in ice, add 50 mL saturated sodium acetate solution, heat on a steam bath for 30 min, cool, filter, recrystallize the solid from EtOH to give 2-hydroxy-4,5-methylenedioxybenzaldehyde as colorless needles (mp 125-126°) (Bull. Chem. Soc. Jpn. 1962, 35, 1321). Stir 1.4 g ethyl 2-chloromethyl-5-oxazolecarboxylate, 1.5 g 2-hydroxy-4,5-methylenedioxybenzaldehyde, 2 g potassium carbonate, and 50 mL anhydrous DMF at 120° overnight, cool, filter. Evaporate the filtrate to dryness under reduced pressure to give 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as a colorless crystalline powder (mp 186°) (yield 39%). Reflux 260 mg 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran, 100 mg KOH, 20 mL EtOH, and 30 mL water for 2 h, concentrate under reduced pressure, dissolve the residue in 100 mL water, wash with ethyl acetate, treat the aqueous layer with activated carbon, acidify the aqueous layer to pH 2 with 2 M HCl. Filter the precipitate and recrystallize it from EtOH to give 2-(2-oxazole-5-carboxylic acid)-5,6-methylenedioxybenzofuran as a colorless crystalline powder (mp 294-295°). Reflux 150 mg 2-(2-oxazole-5-carboxylic acid)-5,6-methylenedioxybenzofuran and 5 mL thionyl chloride for 2 h, pour the reaction mixture into 300 mL petroleum ether. Filter the precipitate and dry it over KOH to give 2-(5-chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran (mp 290°).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Cosmosil 5C18 (Nacalai Tesque)
Mobile phase: MeCN:water 70:30
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 360 em 460

CHROMATOGRAM

Retention time: 4.3
Limit of detection: 750 fmole

OTHER SUBSTANCES

Simultaneous: benzyl alcohol, cyclohexanol, 1-butanol, 1-hexanol, 3-methyl-1-butanol, 2-methyl-2-butanol, 2-methyl-1-propanol, 2-methyl-2-propanol, 1-propanol
Noninterfering: aldehydes, amino acids, aromatic amines, carboxylic acids, ketones, sulfhydryl compounds
Interfering: 2-propanol

KEY WORDS

derivatization

REFERENCE

Nagaoka,H.; Nohta,H.; Kaetsu,Y.; Saito,M.; Ohkura,Y. 2-(5-Chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as fluorescence derivatization reagent for alcohols in high performance liquid chromatography, *Anal.Sci.*, **1989**, *5*, 525–530.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 20 μL aliquot of a solution in 1% acetic acid.

HPLC VARIABLES
Guard column: 30 × 4.6 Spheri-5 RP-18
Column: 250 × 4.6 5 μm Ultrasphere-ODS C18
Mobile phase: Gradient. A was MeCN:acetic acid 99:1. B was 1% acetic acid in water. A:B from 0:100 to 10:90 over 10 min, to 20:80 over 25 min, wash with A for 6 min, re-equilibrate for 14 min.
Flow rate: 2
Injection volume: 20
Detector: F ex 274 em 298

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: catechol (F ex 280 em 325), hydroquinone (F ex 304 em 338), resorcinol (F ex 284 em 313)

REFERENCE
Risner,C.H.; Cash,S.L. A high-performance liquid chromatographic determination of major phenolic compounds in tobacco smoke, *J.Chromatogr.Sci.*, **1990**, *28*, 239–244.

SAMPLE
Matrix: solutions
Sample preparation: Inject 50 μL onto column A in series with column B, after 1.1 min switch so that column A comes after column B, continue to elute.

HPLC VARIABLES
Column: A 15 × 3.2 7 μm New Guard RP-18 (Applied Biosystems); B 100 × 4.6 3 μm Econosphere C18 (Alltech)
Mobile phase: MeCN:water:phosphoric acid 25:75:0.2
Flow rate: 1
Injection volume: 50
Detector: UV 200

CHROMATOGRAM
Retention time: 5
Limit of detection: 0.05 ppm

OTHER SUBSTANCES
Extracted: toluene, cresol, benzoic acid

KEY WORDS
groundwater; water; column-switching

REFERENCE
Chamkasem,N.; Hill,K.D.; Sewell,G.W. High-performance liquid chromatographic column-switching technique for the determination of intermediates of anaerobic degradation of toluene in ground water microcosm, *J.Chromatogr.*, **1991**, *587*, 185–191.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6

Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 5.85 (A), 5.29 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.2 μm) water, remove a 100 μL aliquot and add it to 500 μL 2.59 μM 2-(9-anthryl)ethyl chloroformate in MeCN and 200 μL 25 mM pH 9.6 borate buffer, heat at 43° for 35 min, inject a 10 μL aliquot. (Prepare 2-(9-anthryl)ethyl chloroformate as follows. Stir a solution of 3 g of 9-bromoanthracene in 100 mL ether at 0° under argon or nitrogen, add 9 mL 1.6 M n-butyllithium over 5 min, stir for 30 min, add an ice-cold solution of 3 g ethylene oxide (Caution! Ethylene oxide is a carcinogen!) in 16 mL ether, stir for 1 h, add 70 mL water, add 50 mL ether, remove the organic layer, extract the aqueous layer with 100 mL dichloromethane. Combine the organic layers and wash them with water, dry over anhydrous sodium sulfate, evaporate to dryness, chromatograph on silica gel with dichloromethane to give 2-(9-anthryl)ethanol as pale yellow crystals (mp 106-8°) (J.Org.Chem. 1986, 51, 2956). Stir a solution of 2-(9-anthryl)ethanol in ether in the presence of pyridine (as an HCl scavenger) at 0°, add a solution of phosgene in toluene. 2-(9-anthryl)ethyl chloroformate is obtained as colorless crystals (mp 86-87° from pentane). Protect stock solutions from light and store them in the refrigerator (Anal.Chem. 1991, 63, 292).)

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18

Mobile phase: Gradient. MeCN:water from 70:30 to 100:0 over 10 min, maintain at 100:0 for 10 min
Flow rate: 0.75
Injection volume: 10
Detector: F ex 256 em 418 (cut-off filter)

CHROMATOGRAM
Retention time: 8.57
Limit of detection: 7 nM

OTHER SUBSTANCES
Simultaneous: 4-tert-butylphenol, 3,4-dimethylphenol, 4-methylphenol

KEY WORDS
derivatization; wastewater

REFERENCE
Landzettel,W.J.; Hargis,K.J.; Caboot,J.B.; Adkins,K.L.; Strein,T.G.; Veening,H.; Becker,H.-D. High-performance liquid chromatographic separation and detection of phenols using 2-(9-anthrylethyl) chloroformate as a fluorophoric derivatizing agent, *J.Chromatogr.A*, **1995**, *718*, 45–51.

SAMPLE
Matrix: solutions
Sample preparation: Wash column A with MeOH at 2 mL/min for 1 min, wash column A with 5 mM tetrabutylammonium bromide at 2 mL/min for 1 min, pump sample through column A at 2 mL/min for 2.5 min and elute to waste, backflush the contents of column A on to column B with MeOH for 1 min, remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 2 15-25 μm PLRP-S (Spark Holland); B 250 × 4 ODS-2
Mobile phase: Gradient. MeOH:1% acetic acid from 25:75 to 60:40 over 25 min, to 100:0 over 5 min, maintain at 100:0 for 2 min, return to initial conditions over 2 min.
Column temperature: 65
Flow rate: 1
Injection volume: 5000
Detector: UV 280

CHROMATOGRAM
Retention time: 7
Limit of detection: 0.1-2 ng/mL

OTHER SUBSTANCES
Simultaneous: 4-chloro-3-methylphenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4-dimethylphenol, 2,6-dimethylphenol, 2,4-dinitrophenol, 2-methyl-4,6-dinitrophenol, 2-nitrophenol, 4-nitrophenol, pentachlorophenol (UV 302), 2,4,6-trichlorophenol, 2,4,6-trimethylphenol

KEY WORDS
waste water; river water; column-switching

REFERENCE
Pocurull,E.; Marcé,R.M.; Borrull,F. Improvement of on-line solid-phase extraction for determining phenolic compounds in water, *Chromatographia*, **1995**, *41*, 521–526.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18

Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.79

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.15

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE
Matrix: urine
Sample preparation: Add 1 mL 12 M HCl to 5 mL urine. Heat at 100° for 30 min. Centrifuge at 2500 rpm for 5 min and dilute 40 fold with water. Extract a 1 mL aliquot of this solution with 1 mL isoamyl alcohol saturated with 6 M HCl. Mix for 2 min. Remove a 500 μL aliquot of the organic layer and add it to 500 μL 500 mM NaOH, vortex for 2 min. Inject a 10 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 4.6 10 μm MicroPak RP18
Mobile phase: MeCN:10 mM HCl 20:80
Flow rate: 1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: 11.93
Limit of detection: 50 ng/mL

REFERENCE
Menezes,M.L.; Demarchi,A.C.C.O. Off line extraction of phenol from human urine sample with isoamyl alcohol and determination by HPLC, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 2355–2363.

SAMPLE
Matrix: urine
Sample preparation: Adjust pH to 5, hydrolyze with β-glucuronidase/arylsulfatase at 37° for 12 h, add p-chlorophenol at a final concentration of 50 μg/mL, adjust pH to 2 with HCl. 2 mL Sample + 4 mL dichloromethane, vortex, centrifuge at 2400 g for 15 min. Remove 2.5 mL of the organic layer and add it to 500 μL 200 mM NaOH, vortex. Remove 300 μL of the aqueous layer, adjust pH to 7.0 with HCl, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 33 × 4.6 3 μm Pecosphere 3 × 3 C18
Mobile phase: MeOH:water:orthophosphoric acid 30:70:0.1
Flow rate: 1
Injection volume: 10
Detector: UV 215

CHROMATOGRAM
Retention time: 2.02
Internal standard: p-chlorophenol (8)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: p-nitrophenol, cresols, p-methoxyphenol

REFERENCE
Brega,A.; Prandini,P.; Amaglio,C.; Pafumi,E. Determination of phenol, m-, o- and p-cresol, p-aminophenol and p-nitrophenol in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *535*, 311–316.

SAMPLE
Matrix: urine
Sample preparation: Filter (0.22 μm) urine. Dilute a 100 μL aliquot with 900 μL mobile phase, inject a 200 μL aliquot. Hydrolyze conjugates as follows. 1 mL Urine + 1 mL 200 mM pH 4.8 acetate buffer + 200 μL Helix pomatia juice (containing 100000 Fishman Units of β-glucuronidase and 1000000 Roy Units of sulfatase, IBF, France), heat at 37° overnight, dilute 10-fold with mobile phase, centrifuge at 10000 g for 5 min, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 4.6 10 μm ODS 2
Column: 250 × 4.6 5 μm Sup-Rs Classic ODS 2 (Prolabo)
Mobile phase: MeCN:1% phosphoric acid 10:90
Flow rate: 1
Injection volume: 200
Detector: UV 280

CHROMATOGRAM
Retention time: 20.1
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Extracted: hippuric acid, mandelic acid, 3-methylhippuric acid, phenylglyoxylic acid

REFERENCE
Astier,A. Simultaneous high-performance liquid chromatographic determination of urinary metabolites of benzene, nitrobenzene, toluene, xylene and styrene, *J.Chromatogr.*, **1992**, *573*, 318–322.

SAMPLE
Matrix: urine
Sample preparation: Condition a 500 mg Bond Elut SAX SPE cartridge with 3 mL MeOH and 3 mL water. Dilute 125 μL urine to 4 mL with water, adjust to pH 4.5 with ascorbic acid, add 12.5 μL enzyme solution, heat at 37° for 48 h, add to the SPE cartridge, wash with 3 mL 5 mM pH 7 phosphate buffer. Acidify the eluate to pH <3 with concentrated HCl, add 5 mL ether, vortex, repeat the extraction twice. Combine the organic layers and evaporate them to dryness under reduced pressure at 30°, reconstitute the residue in 1 mL 1% aqueous phosphoric acid, inject a 20 μL aliquot. (The enzyme solutions used to deconjugate glucuronides and sulfate esters were β-glucuronidase/arylsulfatase (Merck, 4114), arylsulfatase (Sigma, S 1629), and β-glucuronidase diluted 1:6 with water (Boehringer, 127051).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil ODS
Mobile phase: MeOH:5 mM pH 3.4 phosphate buffer 30:70
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 9.3
Limit of detection: 36 μg/mL

OTHER SUBSTANCES
Extracted: catechol, hydroquinone

KEY WORDS
mouse; SPE

REFERENCE
Schad,H.; Schäfer,F.; Weber,L.; Seidel,H.J. Determination of benzene metabolites in urine of mice by solid-phase extraction and high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *593*, 147–151.

SAMPLE
Matrix: urine
Sample preparation: Freeze urine, thaw, centrifuge at 6000 g for 10 min, filter (0.45 μm PVDF), dilute with 3 volumes of 40 mM pH 6.8, inject an aliquot onto column A with mobile phase A (phenol conjugates are hydrolyzed to phenol on this column) and elute onto column B, after 10 min remove column A from the circuit and elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 50 × 4.6 immobilized enzyme reactor (Prepare as follows. Stir 1 g Supelclean LC-NH2 (Supelco) in 25 mL 1% glutaraldehyde for 1 h, rinse solid with water and 40 mM pH 6.8 phosphate buffer, stir solid in 25 mL 1 mg/mL β-glucuronidase/sulfatase (type H-2 from Helix pomatia, Sigma) in 40 mM pH 6.8 buffer at 4° for 24 h, wash the solid with water, wash with 100 mM NaCl. Make a slurry of 0.5 g of the immobilized enzyme in 40 mM pH 6.8 buffer and pack in a 50 × 4.6 column using high pressure nitrogen, rinse with 40 mM pH 6.8 phosphate buffer. When not in use store in 100 mM NaCl at 4°.); B 150 × 4.6 5 μm ODS (J & W)
Mobile phase: A MeOH:40 mM pH 6.8 phosphate buffer 30:70; B MeOH:40 mM pH 6.8 phosphate buffer 30:70
Flow rate: 1
Injection volume: 1000
Detector: F ex 270 em 300

CHROMATOGRAM
Retention time: 20
Limit of detection: 10 ppb
Limit of quantitation: 50 ppb

KEY WORDS
column-switching; immobilized enzyme reactor

REFERENCE
Jen,J.-F.; Tsai,M.-Y. Determination of phenol in urine by high-performance liquid chromatography with on-line precolumn enzymatic hydrolysis of the conjugates, *J.Chromatogr.B*, **1994**, *658*, 87–92.

SAMPLE
Matrix: urine
Sample preparation: Filter (0.2 μm), inject an aliquot directly. Hydrolyze conjugates by heating with 6 M HCl at 37° for 18 h, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeOH:1.5% trifluoroacetic acid on water 10:90
Flow rate: 0.5
Detector: UV or radioactivity

CHROMATOGRAM
Retention time: 5.9

OTHER SUBSTANCES
Extracted: metabolites, hydroquinone, phenyl glucuronide, phenyl sulfate

KEY WORDS
rat

REFERENCE
Hughes,M.F.; Hall,L.L. Disposition of phenol in rat after oral, dermal, intravenous, and intratracheal administration, *Xenobiotica*, **1995**, *25*, 873–883.

SAMPLE
Matrix: water
Sample preparation: Add disodium EDTA to sample. 100 mL Water + 10 mL pH 8-9 Britton-Robinson buffer (μ = 0.09) + 1 mL 1.5% 4-aminoantipyrine in water + 5 mL 2% potassium ferricyanide in water + 10 mL chloroform, stir for 10 min, inject a 10 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak phenyl
Mobile phase: MeOH:water 60:40
Flow rate: 1
Injection volume: 10
Detector: UV 480

CHROMATOGRAM
Retention time: k' 1.36
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: 4-chloro-3-methylphenol, 2-chlorophenol, 3-chlorophenol, 4-chlorophenol, 2,3-dichlorophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, 2,3-dimethylphenol, 2,5-dimethylphenol, 2,6-dimethylphenol, 3,5-dimethylphenol, 2-ethylphenol, 3-ethylphenol, 2-methylphenol, 3-methylphenol, 2-nitrophenol, 3-nitrophenol, 2,3,5,6-tetramethylphenol, 2,4,6-trichlorophenol, 2,3,5-trimethylphenol, 2,3,6-trimethylphenol

KEY WORDS
derivatization

REFERENCE
Blo,G.; Dondi,F.; Betti,A.; Bighi,C. Determination of phenols in water samples as 4-aminoantipyrine derivatives by high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *257*, 69–79.

SAMPLE
Matrix: water
Sample preparation: 100 mL Water + 2 mL 50 mM KCl in water + 1 mL 0.6% dextrin in water + 1 mL 80 mM silver nitrate in water + 2 mL pH 9 borax buffer + 500 μL 2% 4-aminoantipyrine in water + 10 mL chloroform, stir at 40° for 40 min, inject an aliquot of the organic layer.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak phenyl
Mobile phase: MeOH:water 60:40
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Limit of quantitation: 0.5 ppm

KEY WORDS
derivatization

REFERENCE
Blo,G.; Dondi,F.; Bighi,C. High-performance liquid chromatographic determination of phenols as 4-aminoantipyrine derivatives; silver chloride as oxidizing agent in the derivatization reaction, *J.Chromatogr.*, **1984**, *295*, 231–235.

SAMPLE
Matrix: water
Sample preparation: Condition a Baker amino SPE cartridge with dichloromethane, dry with nitrogen. Adjust pH to 12 with 1 M NaOH. Remove a 500 μL aliquot and add it to 100 μL 30 mg/mL pH 12 tetrabutylammonium bromide in water, add 500 μL pH 12 water, add 600 μL 100 μg/mL dansyl chloride in dichloromethane, vortex vigorously for 2 min, add a 500 μL aliquot of the dichloromethane layer to the amino SPE cartridge, let stand for 10 min, elute with 3 mL dichloromethane. Evaporate the eluate to dryness, reconstitute the residue in 500 μL MeOH:water 50:50, inject a 100 μL aliquot. (Excess dansyl chloride reacts with the amino groups in the SPE cartridge.)

HPLC VARIABLES
Column: 200 × 3.1 3 μm LiChrosorb RP-18
Mobile phase: Gradient. A was MeOH:100 mM pH 7.0 imidazole buffer 97.5:2.5. B was MeOH: 2.5 mM pH 7.0 imidazole buffer 2.5:97.5. A:B 75:25 for 9.5 min, to 85:15 over 0.5 min, maintain at 85:15 for 4.5 min, to 95:5 over 0.5 min, maintain at 95:5 for 4.5 min, to 100:0 over 0.5 min, maintain at 100:0 for 20 min, return to initial conditions over 1 min, re-equilibrate for 20 min.
Flow rate: 0.5
Injection volume: 100
Detector: Chemiluminescence (470 nm cut-off filter) following post-column reaction. The column effluent flowed through a 130 × 0.3 mm ID PTFE coil irradiated with a fan-cooled 90 w Philips Model 93110E mercury lamp. The effluent from this coil mixed with 50 mM hydrogen peroxide in MeCN containing 5 mM bis(2-nitrophenyl) oxalate pumped at 0.3 mL/min and this mixture flowed to the detector.

CHROMATOGRAM
Retention time: 14
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Simultaneous: 4-chloro-3-methylphenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4-dimethylphenol, 2-nitrophenol, 4-nitrophenol, pentachlorophenol, 2,4,6-trichlorophenol

KEY WORDS
derivatization; post-column reaction; SPE; post-column photochemical derivatization

REFERENCE
Kwakman,P.J.M.; Kamminga,D.A.; Brinkman,U.A.T.; de Jong,G.J. Sensitive liquid chromatographic determination of alkyl-, nitro- and chlorophenols by precolumn derivatization with dansyl chloride, postcolumn photolysis and peroxyoxalate chemiluminescence detection, *J.Chromatogr.*, **1991**, *553*, 345–356.

SAMPLE
Matrix: water
Sample preparation: Prepare an SPE cartridge by adding 500 mg 120-400 mesh Carbograph 4 graphitized carbon black (210 m^2/g, Carbochimica Romana, Rome) to a 65 × 13 polypropylene tube using polyethylene frits. Condition with 10 mL 10 mM tetrabutylammonium chloride in dichloromethane:MeOH 80:20, 2 mL MeOH, and 14 mL water acidified to pH 2 with HCl. Filter (Whatman GF/C 1.5 μm glass fiber) river water, pass 4 L through the SPE cartridge at 100 mL/min, wash with 7 mL water at 5-7 mL/min, pull air through the SPE cartridge for 1 min, wash with 800 μL MeOH, dry under vacuum for 1 min, elute in a reverse fashion with 6 mL 10 mM tetrabutylammonium chloride in dichloromethane:MeOH 80:20 at 6 mL/min. Remove a 3 mL aliquot of the eluate and evaporate it to dryness under a stream of nitrogen at 27°, reconstitute the residue in 150 μL 100 mM sodium carbonate in MeCN:water 20:80, add 40 μL acetic anhydride, heat at 50° for 6 min, inject a 50 μL aliquot. (Phenols can also be determined without derivatization. Derivatization provides confirmation of peak identity.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Alltima LC-18 (Alltech)
Mobile phase: Gradient. A was 0.025% trifluoroacetic acid in water. B was 0.0125% trifluoroacetic acid in MeCN. A:B from 78:22 to 10:90 over 27 min.
Flow rate: 1
Injection volume: 50
Detector: UV 280 for 18 min then UV 220

CHROMATOGRAM
Retention time: 12.4
Limit of detection: <50 ng/mL

OTHER SUBSTANCES
Simultaneous: 4-chloro-3-methylphenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4-dimethylphenol, 4,6-dinitro-2-methylphenol, 2,4-dinitrophenol, 2-nitrophenol, 4-nitrophenol, pentachlorophenol, 2,4,6-trichlorophenol

KEY WORDS
derivatization; SPE; river water

REFERENCE
Di Corcia,A.; Bellioni,A.; Madbouly,M.D.; Marchese,S. Trace determination of phenols in natural waters. Extraction by a new graphitized carbon black cartridge followed by liquid chromatography and re-analysis after phenol derivatization, *J.Chromatogr.A*, **1996**, *733*, 383–393.

SAMPLE
Matrix: water
Sample preparation: Condition a 500 mg ENVI Chrom P highly cross-linked styrene-divinylbenzene SPE cartridge (Supelco) with 10 mL MeOH, 10 mL water, and 2 mL 5 mM tetrabutylammonium bromide, dry. Filter (0.45 μm) sample, add 3 mL 10% sodium sulfite solution to each 1 L of water, adjust pH to 9 with 1 M NaOH, add tetrabutylammonium bromide to a final concentration of 5 mM, pass a 500 mL aliquot through the SPE cartridge, elute with 5 mL MeOH, acidify with 1% acetic acid, evaporate to 1 mL under reduced pressure, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Spherisorb ODS-2
Mobile phase: Gradient. MeOH:1% pH 2.8 acetic acid from 25:75 to 40:60 over 25 min, to 100:0 over 5 min, maintain at 200:0 for 2 min, return to initial conditions over 2 min
Column temperature: 65
Flow rate: 1
Injection volume: 20
Detector: UV 280

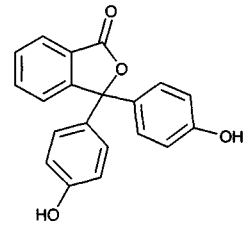
CHROMATOGRAM
Retention time: 6
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Simultaneous: other phenolic compounds

KEY WORDS
river water; SPE

REFERENCE
Pocurull,E.; Calull,M.; Marcé,R.M.; Borrull,F. Determination of phenolic compounds at low μg l^{-1} levels by various solid-phase extractions followed by liquid chromatography and diode-array detection, *J.Chromatogr.A*, **1996**, *719*, 105–112.

Phenolphthalein



Molecular formula: $C_{20}H_{14}O_4$
Molecular weight: 318.33
CAS Registry No.: 77-09-8
Merck Index: 7392

SAMPLE
Matrix: bile, blood, urine
Sample preparation: Condition a 1 mL Bond-Elut C18 SPE cartridge with 2 column volumes of MeOH and 2 column volumes of water, do not allow to go dry. Serum. 1 mL Serum + 25 μL 400 μg/mL bromocresol purple in water + 3 mL acidified acetone, vortex for 5 min, centrifuge at 1200 g for 10 min. Remove 3 mL of the supernatant and evaporate it to dryness under reduced pressure, reconstitute the residue in 1 mL 100 mM pH 7.4 sodium phosphate buffer containing 0.9% NaCl, sonicate for 10 min, add to the SPE cartridge, wash with 2 column volumes of water, allow to dry. Elute with two 800 μL aliquots of MeOH, evaporate the eluate to dryness under reduced pressure, reconstitute in 1 mL MeCN:50 mM NaH_2PO_4 10:90, centrifuge at 1200 g for 10 min, inject a 100 μL aliquot. Urine. 100 μL Urine + 25 μL 400 μg/mL bromocresol purple in water + 875 μL 100 mM pH 7.4 sodium phosphate buffer containing 0.9% NaCl, mix, add 3 mL acetone:water 88:12, vortex for 5 min, centrifuge at 1200 g for 10 min. Remove 3 mL of the supernatant and evaporate it to dryness under reduced pressure, reconstitute the residue in 1 mL 100 mM pH 7.4 sodium phosphate buffer containing 0.9% NaCl, sonicate for 10 min, add to the SPE cartridge, wash with 2 column volumes of water, allow to dry. Elute with two 800 μL aliquots of MeOH, evaporate the eluate to dryness under reduced pressure, reconstitute in 1 mL MeCN:50 mM NaH_2PO_4 10:90, centrifuge at 1200 g for 10 min, inject a 100 μL aliquot. Bile. 50 μL Bile + 950 μL MeCN:50 mM NaH_2PO_4 10:90, centrifuge at 1200 g for 10 min, inject a 50 μL aliquot of the supernatant. (Acidified acetone was 0.5 mL glacial acetic acid in 880 mL acetone, made up to 1 L with water.)

HPLC VARIABLES
Guard column: 5.3 × 4 10 μm Bondapak C18 guard-pak
Column: 150 × 4.6 5 μm LC-18DB (Supelco)

Mobile phase: Gradient. A was MeCN:50 mM NaH_2PO_4 10:90. B was MeCN:50 mM NaH_2PO_4. A:B 65:35 for 5 min, to 40:60 over 0.1 min, maintain at 40:60 for 8 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 1
Injection volume: 100
Detector: UV 230

CHROMATOGRAM
Retention time: 11.4
Internal standard: bromocresol purple (9.5)
Limit of detection: 10 μg/mL (bile), 500 ng/mL (urine), 100 ng/mL (serum)

OTHER SUBSTANCES
Extracted: metabolites, glucuronides

KEY WORDS
serum; dog; SPE; pharmacokinetics

REFERENCE
Wilhelm,J.A.; Bailey,L.C.; Shepard,T.A.; Venturella,V.S. Simultaneous determination of phenolphthalein and phenolphthalein glucuronide from dog serum, urine and bile by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *578*, 231–238.

SAMPLE
Matrix: blood, tissue
Sample preparation: Tissue. Homogenize tissue with 2 volumes of water. 1 mL Homogenate + 300 μL MeOH + 1 mL buffer + 10 mL dichloromethane, rotate, centrifuge. Remove the organic layer and add it to 3 mL 100 mM NaOH, rotate, centrifuge. Remove the aqueous layer and acidify it with 1 mL 1 M HCl, extract with 10 mL dichloromethane. Remove the organic layer and evaporate it to dryness at 40°, reconstitute the residue in 300 μL MeOH, inject a 20 μL aliquot. Blood. 500 μL Whole blood + 500 μL buffer + 200 μL MeOH + 5 mL dichloromethane, rotate, centrifuge. Remove the organic layer and evaporate it to dryness at 40°, reconstitute the residue in 300 μL MeOH, inject a 20 μL aliquot. (Buffer was prepared by mixing 500 mM Na_2HPO_4 with 500 mM KH_2PO_4 to pH 5.5.)

HPLC VARIABLES
Column: 250 × 4.6 10 μm RP-8 (Hewlett-Packard) or 260 × 4.6 10 μm Spherisorb C18
Mobile phase: MeOH:water 60:40
Flow rate: 2
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 2.96
Internal standard: phenolphthalein

OTHER SUBSTANCES
Extracted: thiopental
Simultaneous: carbamazepine, pentobarbital (UV 210)
Noninterfering: amobarbital, butabarbital, glutethimide, meprobamate, methaqualone, methyprylon, phenobarbital, phenytoin, secobarbital

KEY WORDS
phenolphthalein is IS; whole blood

REFERENCE
Levine,B.; Blanke,R.; Valentour,J. Liquid chromatographic analysis of thiopental in blood and tissues, *J.Anal.Toxicol.*, **1983**, *7*, 207–208.

SAMPLE
Matrix: formulations

Sample preparation: Weigh out formulation containing 500 mg phenolphthalein, make up to 100 mL with MeOH, sonicate for 5 min, filter (0.45 μm). Dilute 5 mL of the filtrate to 100 mL with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4 7 μm Nucleosil C18
Mobile phase: MeOH:water:acetic acid 50:50:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 3.9
Limit of detection: 4 μg/mL

OTHER SUBSTANCES
Simultaneous: aloin

KEY WORDS
granules; tablets; dragees; emulsions; comparison with spectrophotometric method

REFERENCE
Torrado,S.; Fraile,S.; Torrado,J.J.; Selles,V.E. Comparison of reversed-phase liquid chromatography with colorimetry for analysis of phenolphthalein preparations, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1167–1172.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 5 μm LiChrospher CH-18
Mobile phase: MeOH:10 mM pH 6.0 phosphate buffer 75:25 containing 2.5 mM cethexonium bromide (Rinse with 100 mL MeOH:EtOH:water 50:25:25 at the end of the day.)
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.3

OTHER SUBSTANCES
Extracted: glucuronides

REFERENCE
Liu,H.-F.; Leroy,P.; Nicolas,A.; Magdalou,J.; Siest,G. Evaluation of a versatile reversed-phase high-performance liquid chromatographic system using cethexonium bromide as ion-pairing reagent for the analysis of glucuronic acid conjugates, *J.Chromatogr.*, **1989**, *493*, 137–147.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS (Altex)
Mobile phase: MeOH:water 5:1
Flow rate: 2
Injection volume: 20
Detector: UV 290

OTHER SUBSTANCES
Simultaneous: thiamylal

REFERENCE
Costantino,A.G.; Caplan,Y.H.; Levine,B.S.; Dixon,A.M.; Smialek,J.E. Thiamylal: review of the literature and report of a suicide, *J.Forensic Sci.*, **1990**, *35*, 89–96.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 6.05 (A), 5.66 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

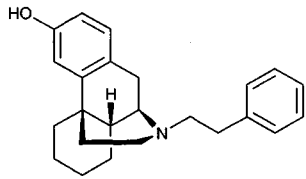
CHROMATOGRAM
Retention time: k' 0.96

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

Phenomorphan



Molecular formula: $C_{24}H_{29}NO$
Molecular weight: 347.50
CAS Registry No.: 468-07-5
Merck Index: 7399
Lednicer No.: 1 294

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.3

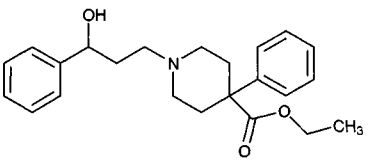
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, er-

gosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Phenoperidine



Molecular formula: $C_{23}H_{29}NO_3$
Molecular weight: 367.49
CAS Registry No.: 562-26-5, 3627-49-4 (HCl)
Merck Index: 7400
Lednicer No.: 1 302

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.7

OTHER SUBSTANCES

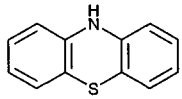
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine,

chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Phenothiazine



Molecular formula: $C_{12}H_9NS$
Molecular weight: 199.28
CAS Registry No.: 92-84-2
Merck Index: 7404

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 204

CHROMATOGRAM
Retention time: 14.122, 14.265

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Evaporate reaction mixture (if necessary, chromatograph residue on deactivated alumina with MeOH), dissolve residue in MeOH with IS, inject an aliquot.

HPLC VARIABLES
Column: 150 × 5 Spherisorb A5Y alumina
Mobile phase: Hexane:ethyl acetate containing 0.6% water 95:5
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.32
Internal standard: 4-methoxy-2-nitroaniline (k' 8.60)

KEY WORDS
normal phase

REFERENCE
Lunn,G. *Nitrogen-containing Reactive Intermediates in Heterocyclic Synthesis*, Ph.D. Thesis, University of Edinburgh, **1975**.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.0

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquin-

amide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 70:1.5:0.5:28
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 1.56

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Mix 100 µL of a 0.05-1 µg/mL solution in MeOH with 20 µL 5% sodium carbonate and about 35 mg of a suspension of W-2 Raney nickel in EtOH (80 µL), stir at 70° for 15 min, cool in ice-water, add 50 µL 400 ng/mL 1-naphthol in MeOH, mix well, filter (0.45 µm), inject a 5 µL aliquot of the filtrate. (Raney nickel can be purchased from Aldrich or

prepared from aluminum-nickel alloy (Org. Syn. 1955, Coll. Vol. 3, 181). Phenothiazine is desulfurized to diphenylamine.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Shim-pack CLC-ODS (Shimadzu)
Mobile phase: MeOH:water 75:25 containing 50 mM sodium perchlorate and 5 mL/L glacial acetic acid
Column temperature: 35
Flow rate: 0.6
Injection volume: 5
Detector: E, Showa Denko Shodex EC-1, glassy carbon working electrode 0.8 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 12.5
Internal standard: 1-naphthol (8.5)
Limit of detection: 10 pg

KEY WORDS
derivatization; desulfurization

REFERENCE
Shimada,K.; Mino,T.; Nakajima,M.; Wakabayashi,H.; Yamato,S. Application of the desulfurization of phenothiazines for a sensitive detection method by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *661*, 85–91.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron) 1 g tissue + 5 mL MeOH, centrifuge at 1600 g for 5 min. Remove 1 mL of the supernatant and evaporate it to dryness, reconstitute the residue in 1 mL cyclohexane, centrifuge, inject a 50 μL aliquot of the supernatant.

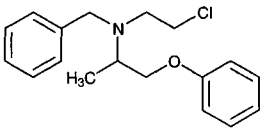
HPLC VARIABLES
Guard column: 30 × 2.8 30 μm pellicular beads
Column: 250 × 4.6 Partisil-10
Mobile phase: Cyclohexane:n-propanol 99.7:0.3
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 9
Limit of detection: 50 ppb

KEY WORDS
sheep; kidney; muscle; liver; fat

REFERENCE
Blackman,G.L.; Ho,A.C.; Jozsa,A.; Kelly,J.D. High performance liquid chromatographic determination of phenothiazine residues in sheep tissues, *J.Assoc.Off.Anal.Chem.*, **1980**, *63*, 988–991.

Phenoxybenzamine



Molecular formula: $C_{18}H_{22}ClNO$
Molecular weight: 303.83
CAS Registry No.: 59-96-1, 63-92-3 (HCl)
Merck Index: 7409
Lednicer No.: 1 55

SAMPLE

Matrix: blood, tissue
Sample preparation: Homogenize tissue with 3 parts of 50 mM pH 7.4 phosphate buffer, sonicate adipose tissue homogenates for 30 s. 1 mL Plasma or tissue homogenate + 500 μL 500 mM sodium carbonate + 6 mL ethyl acetate, extract for 1 h, centrifuge. Remove the organic layer and dry it by mixing with anhydrous sodium sulfate for 20 s, centrifuge, evaporate 5 mL to dryness under a stream of nitrogen at 50°, reconstitute the residue in 500 μL n-heptane, add 200 μL MeOH:1 M HCl 90:10, mix for 4 min, centrifuge, discard the heptane layer, inject a 10 μL aliquot of the MeOH/HCl layer.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb ODS2
Mobile phase: MeCN:MeOH:10 mM pH 8.0 phosphate buffer 20:66:14
Flow rate: 1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM

Limit of detection: 10 ng

KEY WORDS

rat; plasma; liver; kidney; lung; brain; heart; muscle; adipose tissue

REFERENCE

Moor,M.J.; Bickel,M.H. Tissue distribution of phenoxybenzamine in the rat. Lack of adipose tissue storage, *Life Sci.*, **1987**, *41*, 2041–2046.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.0

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextro-

propoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 1.78

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

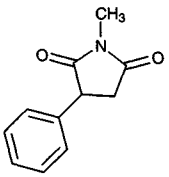
Column: 250 × 4.6 cellulose tris(3,5-dimethylphenylcarbamate)
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 0.5
Detector: UV

CHROMATOGRAM
Retention time: k' 0.70 (of first (-) enantiomer)

KEY WORDS
chiral; α 1.13

REFERENCE
Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

Phensuximide



Molecular formula: $C_{11}H_{11}NO_2$
Molecular weight: 189.21
CAS Registry No.: 86-34-0
Merck Index: 7414
Lednicer No.: 1 226

SAMPLE
Matrix: solutions
Sample preparation: Inject a 6-10 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM
Retention time: 4.22
Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetanilide, N-acetylcysteine, N-acetylprocainamide, amobarbital, ampicillin, aspirin, barbital, butalbital, caffeine, carbamazepine, chloramphenicol, chlorpropamide, cimetidine, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, ethosuximide, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, mephobarbital, methaqualone, methsuximide, methyl salicylate, methyprylon, morphine, naproxen, nirvanol, oxphenylbutazone, pentobarbital, phenacetin, phenobarbital, phenylbutazone, phenytoin, primidone, procainamide, salicylamide, salicylic acid, secobarbital, sulfamethoxazole, sulindac, theophylline, thiopental, tolmetin, vancomycin
Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid
Interfering: butabarbital, trimethoprim

REFERENCE
Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4.6 3 μm 208HS3410 (Vydac)
Mobile phase: Gradient. MeCN:water from 15:85 to 60:40 over 10 min.
Flow rate: 1.5
Detector: UV 210 (?)

CHROMATOGRAM
Retention time: 5.2

OTHER SUBSTANCES
Simultaneous: barbital, carbamazepine, diazepam, ethotoin, mephenytoin, methsuximide, phenacemide, phenobarbital

REFERENCE
Vydac HPLC Catalog, 1994-5, **1994**, p. 26.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

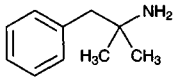
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyril-

amine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Phentermine



Molecular formula: $C_{10}H_{15}N$
Molecular weight: 149.24
CAS Registry No.: 122-09-8, 1197-21-3 (HCl)
Merck Index: 7415
Lednicer No.: 1 72

SAMPLE
Matrix: bulk
Sample preparation: Mix a 1 mg/mL solution in 1 M sodium carbonate with 2 mL 5 mg/mL 8-quinolinesulfonyl chloride in acetone, heat at 65° for 20 min, cool, extract twice with 30 mL portions of chloroform. Combine the extracts and dry them over anhydrous magnesium sulfate, evaporate to dryness under a stream of air, reconstitute, inject an aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 Co:Pell ODS
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 40:59:1
Flow rate: 1.5
Detector: UV 254, UV 280

CHROMATOGRAM
Retention time: 30

OTHER SUBSTANCES
Simultaneous: amphetamine, ephedrine, methamphetamine, phenmetrazine, phenylpropanolamine, pseudoephedrine

KEY WORDS
derivatization

REFERENCE

Noggle,F.T.,Jr.; Clark,C.R. Liquid chromatographic determination of primary and secondary amines as 8-quinolinesulfonyl chloride derivatives, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 687–691.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject 75-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco